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Histologic Study of a Re-Entry Wound with Periosteal Preservation

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**HISTOLOGIC STUDY OF A RE-ENTRY WOUND
WITH PERIOSTEAL PRESERVATION**

By

PETER DITTMAR ROBERSON

**A Thesis Submitted to the Faculty of the Graduate
School of Loyola University in Partial Fulfillment
of the Requirements for the Degree of Master of Science.**

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Dedication

For their patience, understanding and loyalty during the preparation of this manuscript, this thesis is dedicated to my fiancée' and to my family.

PDR

Acknowledgments

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L I F E

Peter Dittmar Roberson was born September 25, 1936, in Chicago, Illinois.

He received his secondary education at Loyola Academy, and was graduated in June, 1954. That same year he entered the University of Notre Dame. He received his Bachelor of Science Degree from Notre Dame in June, 1958. After one year in the Graduate School of Loyola University, Chicago, he enrolled in the Dental School of Loyola University, receiving the degree of Doctor of Dental Surgery in June, 1963.

Upon graduation the author served for two years as an officer in the United States Navy. One year of his service was spent with the United States Marine Corps in the Far East, including a tour of duty in Viet Nam.

In 1965, the author started his advanced training in Periodontics at Loyola University, Chicago, Illinois.

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CHAPTER I

Introduction

An understanding of the biologic phenomenon occurring during the healing of gingival wounds has evolved with a greater awareness of cellular mechanizations which are necessary for repair of the many and varied types of wounds which are now created in clinical periodontics.

Recently, investigations were undertaken to determine the cellular response to surgically induced wounds. It has been determined that cells react in a predictable manner. In a pilot study of wound healing, it has been determined that cellular mobilization and subsequent repair is enhanced by a two-phase or so-called re-entry surgical procedure.

This study is proposed to elucidate the findings already gained from previous experiments in wound healing, and to apply this knowledge to a surgical procedure that is known to be traumatic and detrimental to overall repair of the periodontium. In so doing it is hoped that by using cellular mechanisms already present in an actively healing wound, a second re-entry and more traumatic wound will not adversely affect the ultimate result.

CHAPTER II

Review of Literature

I. Introduction.

Until recent times the stated objectives of periodontal surgical procedures were the elimination of the periodontal pockets, and the preservation of supporting alveolar bone. It was to these ends that the first research studies of a therapeutic nature were directed. The findings from these studies, therefore, led researchers in periodontal surgical procedures to still another objective, the preservation of mature masticatory mucosa.

Now one finds in the periodontal literature investigations pertaining to the shifting of mature masticatory mucosa from one level to another. These include "repositioning the attached gingiva" by Nabers (1954) and the variations of mucogingival flap surgery which preserve the majority of the mature masticatory mucosa, by Freidman (1962), Ochsenbein (1963), and Morris (1965). The most current investigations pertain to the engraftment of mature masticatory mucosa, Nabers (1966), for the correction of mucogingival periodontal pathologies.

However, it is the clinical and histological research findings pertaining to the preservation of and the minimizing

of trauma to the supporting alveolar bone, utilizing mucogingival flap procedures, that is of interest as a prelude to the presented investigation.

II. Clinical Studies.

In 1947 Dingman utilized humans to study mucoperiosteal flaps. He found it was permissible to detach gingival tissue from around the teeth, and that in seven to ten days it was clinically impossible to determine that the gingival tissue had been disturbed.

The impetus for the histologic investigations of wound healing came from clinical muco-gingival procedures that eliminated the periodontal pocket but left exposed portions of alveolar bone (Fox, Schluger, 1956).

Freidman (1957) studied the healing of muco-gingival resections. He reported that gingival healing starts at the periphery of the wound and from the crest of the bone, with the majority of the new gingival tissue originating from the alveolar bone.

However, leaving alveolar bone exposed was considered undesirable so surgical methods were sought to protect the alveolar bone.

Ochsenbein, in 1960, advocated the use of a double flap. In this procedure, a muco-gingival flap was reflected and then the periosteum was raised from the alveolar bone. Subsequent to osseous procedures the periosteum would be replaced over the

bone to cover and protect it.

J. J. Carrano, et al, (1964) performed a clinical mucogingival comparative study on seven patients. They denuded mandibular alveolar bone on one side and left periosteal covered alveolar bone on the other. Their results indicate that the post-operative width of the gingiva is greater when the periosteum is left intact; and that there is no relation between the width of denuded bone and post-operative gingival width.

III. Histologic Studies.

The groundwork was first laid by Orban and Archer in 1945. Histologic healing of a gingivectomy wound in humans was studied. It was noted that complete epithelization took place in fourteen days. Also, the significance of the blood clot was of great importance.

Further investigation of the healing and repair of human gingival tissues were made by Benier and Kaplan in 1947. They found that a gingival wound was covered by stratified squamous epithelium in six days, with an almost normal periodontal ligament and attachment of the alveolar crest gingival fibers to the alveolar bone, also present at this time. Specimens of sixteen-day-old wound gave evidence of mature epithelium and a dense collagenous connective tissue. These results, the authors stressed, were obtainable because a surgical dressing

was placed on the exposed wound which facilitated healing. Also, in 1947 Workman concluded that by four weeks post-operatively, the tooth and periodontal ligament relationship was restored to its pre-operative dimension.

A study of dogs and humans by Borden in 1948 showed that (a) connective tissue fibers of the periodontal membrane will re-attach themselves to the cementum; (b) new cementum, bone and connective tissue fibers were not essential for reattachment; and (c) granulation tissue formed in the periodontal space with subsequent fibrosis at the site of separation.

Grant and Ivancie in 1957 found that the new gingival tissue which formed after a gingival repositioning procedure, was considered to be functional, though histologically immature, and less differentiated than the control tissue.

Douglas, in 1958, using the works of Sandbloom (1949), Taffel (1951), and Sovluy and Dunphy (1954) as a basis, studied the tensile strength of wounds. It was shown that if (a) an incision wound was made, closed with sutures and left to heal; (b) re-wounded in a similar fashion at a later time, closed and sutured again; the tensile strength of the resutured wound was greater than that of the fresh wounds at all experimental time intervals. At a twenty-one day interval between woundings, the maximal cellular effect was seen.

The effect of repeated injuries to the gingiva of rats was studied by Stahl (1962). He found that the repeated

wounds epithelized one week earlier than the first injury, and that re-injury of an already healed wound did not affect the ultimate repair potential.

Glickman, et al (1963), utilizing dogs in a comparative histologic study of a periosteum retained, and of a periosteum removed muco-gingival wound, reported: "removing of periosteum during muco-gingival surgery delayed healing and caused a significant loss in the height of the alveolar bone." The authors also noted several interesting facets in the periosteal retained wound: (a) there was a slight net loss in alveolar bone height; (b) the epithelium lacked the normal rete peg formation; and (c) the connective tissue repaired as a scar without a clean-cut papillary layer.

Toto and Abati (1963) used tritiated thymidine to measure the uptake of D.N.A. In order to localize the source of cells in granulation tissue. The authors reported that the connective tissue reserve cells (perivascular reserve cells) are the ones that undergo mitosis and differentiation.

Utilizing human block sections, Pfeifer (1965) studied the reactions of alveolar bone to flap procedures, muco-periosteal and split thickness. The author summarized his findings which confirm previous animal studies:

1. Osteoclastic activity occurred in the apically repositioned flap specimens in the crestal area and along the gingival tissue surface of the alveolar process.

2. Very little osteoclastic activity was seen in the split flap procedures where a thick layer of tissue was left covering the bone. The activity that was seen was limited to the apex of the incisions. Severe osteoclastic activity was seen in those areas where the bone was nicked in preparation of the flap and where the periosteum was inadvertently removed.
3. Only in two of the seven specimens was osteoclastic activity seen on the periodontal ligament surface of the alveolar bone.
4. The gingival tissues healed and matured rapidly in both procedures.

Wilderman and Wentz (1965) studied the histologic repair of a created dento-gingival defect with a pedicle flap. Of particular interest is the author's observations regarding osteoclasia, and osteogenesis. The authors state that osteoclastic activity occurs between four and fourteen days following the reparative surgery. The greatest osteoblastic activity is seen in the twenty-one and twenty-eight day post-operative specimen.

In a two-part study, Ramfjord and his associates in 1966 utilized tritiated thymidine to study epithelial and connective tissue healing of a simple gingivectomy wound. Regarding epithelial repair the authors state that the migrating epithelial cells start to cover the wound between the twelfth and twenty-fourth post-operative hour and although the surface of the wound appears to be healed in two weeks, complete healing of the gingival sulcus takes four to five weeks.

Connective tissue proliferation is initiated one to two days post-operative and peaks at three to four post-operative days. Functional and physiologic healing of the dento-gingival junction is accomplished in three to five post-operative weeks.

IV. Studies Directly Pertinent to this Investigation.

Kollar, et al (1955) air abraded the attached gingiva in humans in order to study the rate of re-epithelization. The authors reported mitotic activity was most prominent at the twenty-four hour period following injury and that complete repair occurred between twenty-four and forty-eight hours after injury.

Mittelman in 1958 investigated the repair of a stab wound. He found epithelialization in twenty-four hours with residual inflammation in the connective tissue at seventy-two hours, which led him to the conclusion that epithelial regeneration is more rapid than connective tissue regeneration.

Marfino (1958) investigated the healing of a flap procedure in which 6 x 7 mm crestal alveolar bone was removed. Healing of the wound occurred with new crestal bone but without total repair to the pre-operative level.

Wilderman (1959) by exposing the vestibular alveolar bone via a muco-periosteal flap, showed healing that resulted in a deficiency in the repair of the vestibular alveolar bone. The connective tissue apparatus increased in height to compensate

for that deficiency and the epithelial attachment was in a more apical position. In other words, the periodontal complex healed functionally, but with an anatomical deformity.

A study by Staffileno (1961) carried Wilderman's work a step further. Staffileno utilized a partial thickness flap which in effect left a wound in which there was no epithelial attachment and only a thin layer of connective tissue, fibrous periosteum covering the vestibular alveolar bone. The partial thickness flap then was reapposed to its original position and sutured to place. Healing of this wound revealed that the dimensions of distance from the alveolar crest to the epithelial attachment and the length of the fibrous connective tissue attachment remained at the pre-operative control level. Staffileno concluded the wound healed with no anatomic deformity.

West (1961) also reported on wounds in which periosteum was retained. He found that the vestibular bone was partially resorbed and that repair was rapid.

Wilderman (1963) studied histologically the repair of the oral mucosa and the dento-gingival junction in dogs. A measured partial thickness flap similar to Staffileno's was reflected, excised at its apical base, and discarded. The remaining alveolar mucosa was then sutured to its underlying tissue. However, a notch was made in bone apically to the alveolar bone crest, at the base of the wound. Osteoclastic activity was seen to begin in the crestal area im-

mediately, and reached a peak between the fourth and sixth days, post-operatively. Osteoclastic activity was seen in the notch area twenty-eight days post-operatively. It was further reported that epithelial migration began from the wound edges and completely covered the wound in ten days. Connective tissue repair was seen to originate and proliferate from the tissues at the sites of bone resorption. The greatest osteogenic activity was seen to occur in the crestal area between fourteen and twenty-one days post-operatively.

Giblin (1964) conducted an experiment to determine the effect of re-wounding a periosteal retention mucogingival flap. His flap procedure was similar to Staffileno's. However, at twenty-one days after reflecting the initial flap the same flap was again raised and then re-sutured. Animal sacrifice was initiated at various time intervals following the re-wounding. From the histologic findings Giblin concluded:

1. Epithelial repair following a second surgical injury is much more rapid than that following an initial surgical injury;
2. Connective tissue repair is neither accelerated nor enhanced by initiation of a second surgical injury;
3. Localized resistance to osteoclastic resorption is greater to a second surgical injury than to initial surgical injury;

4. The noted localized resistance to the osteoclastic resorption of a second surgical injury results from the first surgical procedure which induces the apposition of a resorption resistant osteoid layer and the development of a protective dense fibroperiosteal connective tissue layer.

Staffileno et al in 1966 reported on a histologic study similar to the one Wilderman conducted in 1963. However, the alveolar bone was not notched at the base of the flap. The authors reported a complete functional repair with a slight anatomical deformity of the dento-gingival junction; and concluded that a periosteal retention mucogingival flap results in:

- "1. Minimal tissue destruction;
2. Rapid repair;
3. Slight alteration of the dentogingival junction and maximum preservation of periodontal supporting structures."

CHAPTER III

Material and Methods

The experimental procedures were performed on six adult mongrel canines, five males and one female. The animals were approximately two and one-half years of age and had an average weight of twenty-three kilograms. The oral cavities were normal.

General anesthesia, pentobarbital sodium, was used for all surgical procedures, including an overdose amount for animal sacrifice. The material and dosage used for surgical anesthesia was Diabutal*, sixty milligrams per milliliter per kilogram of animal weight. The anesthetic was injected intravenously via the cephalic vein. Animal sacrifice was achieved by total tox (Chicago Veterinary Supply House) at a dosage of 325 mg per milliliter.

The vestibular right and left mandibular pre molar areas were the sites used for surgical investigation. The investigation was conducted in five phases:

Phase I - The first surgical procedure. (Fig. 2a).

The animal was weighed, anesthetized, and prepared for

*Diamond Laboratories, Iowa.

surgery. Sterile techniques were utilized at all times. The operative site was washed with zephirin chloride, 1:1000 dilution. A diamond disc was used to notch the enamel and dentin of the second, third and fourth mandibular premolar teeth at the crest of the free gingival margin so as to give a fixed reference point, and therefore a means of measuring the height of the alveolar bone and the post-surgical dento-gingival junction.

The surgical procedure consisted of laying a vestibular partial thickness flap by making two oblique-vertical incisions, one mesial to the second mandibular premolar and the other distal to the fourth mandibular premolar. These incisions were made to the bone. A horizontal incision was then made into the free gingiva, at the point of the distal oblique-vertical incision and was carried across the teeth and interdental spaces to the mesial oblique-vertical incision point. This freed the gingiva from the teeth. The horizontal incision was carried on into the deep layers of the lamina propria, thus freeing the attached gingiva and lamina propria from the periosteal covered alveolar bone. The partial thickness flap thus raised, measured for the six animals ten millimeters by forty-three millimeters, and allowed the alveolar bone to be protected by periosteal connective tissue at all times. Portions of the crevicular epithelium and epithelium attachment were left unsutured and adhered to the teeth. The flap was reapposed and immobilized

with triple "0" silk sutures. At no time was the alveolar bone touched while the horizontal incision was being made.

The wounds were not dressed in any manner. The animals were given oxytetracycline 25 mg per kilogram of body weight, intravenously immediately after the surgical procedure.

The animals were put on a diet of Wayne Dog Food* served in a very soft consistency. This completed Phase I of the initial surgical procedure.

Phase II - Intermediate Healing Period.

Sutures were removed on the seventh day following surgery. Following the first surgical procedure, the surgical sites were allowed to recover for a twenty-one day period. This completed the second phase of the procedure.

Phase III - The Second Surgical Procedure (Re-Entry Surgery) (Fig. 26).

On the twenty-first day following initial surgery, the same operative site was prepared for re-entry surgery. The same sterile conditions were observed. Using the scars from the previous surgery as a guide, a periosteal preservation type of flap was raised, dissected away, and discarded, thus leaving a large wound exposing the periosteum and some connective tissues covering the previous wound site.

The base of the wound was in the alveolar mucosa superior to the vestibular fold. The alveolar bone was covered by periosteum, and a thin layer of connective tissue. The

*Allied Mills, Inc.

alveolar bone was not touched during flap dissection. As in the first surgery, portions of crevicular epithelium and epithelial attachment were left adhering to the teeth. Hemorrhage was controlled by applying sterile gauze packs with light pressure over the wound for several minutes. The wounds were not dressed as in the initial surgery. The animals were given Demethyl-chlortetracycline* 50 mg. per kilogram of body weight, orally, several hours prior to the re-entry procedure and on every other day until the twenty-first day post-operative. The same diet of Wayne Dog Food was given the animals. This completed the third phase of the procedure and also the surgery.

Phase IV - Wound Healing.

In this phase of the investigation the animals were allowed to survive to the designated time intervals for sacrifice. The lingual side of each experimental area was used as a control site.

Phase V - Animal Sacrifice.

Following the second surgical entry, the animals were sacrificed at the following time intervals:

<u>Time from initial wound</u>	<u>Re-entry wound and sacrifice times</u>
21 days	0 days
22 "	1 "
23 "	2 "
24 "	3 "
28 "	7 "
35 "	14 "
42 "	21 "
51 "	30 "

*Declomycin, Lederle Labs, Inc.

Time from initial wound

Re-entry wound and
sacrifice times

81 days
111 "
141 "
201 "

60 days
90 "
120 "
180 "

The animals were sacrificed by an overdose of Pentobarbital Sodium; the tissue specimens were excised from the animals in block sections. The specimens were washed in running water and placed in fresh ten-percent neutral formalin solution.

Preparation of Histologic Sections.

The tissue specimens were allowed to be fixed in ten-percent neutral formalin for two weeks. The solutions were changed twice during that time. After a two-week fixation period, the block sections were trimmed for histologic preparation.

The mandibular third premolar area was selected for this investigation, for this area was the most central, and had the least handling during removal from the animal. The specimens were decalcified in sodium citrate and formic acid solution which was changed on every third day. Radiographs were used to determine the degree of decalcification.

Tissue embedding in paraffin was accomplished after dehydration of the specimens in ascending concentrations of alcohol. The tissue specimens were sectioned serially, oral vestibularly at ten microns.

Histologic staining was accomplished with hematoxylin-eosin

27.
silver impregnation and both Mallory's and Heidenhain's
connective tissue stain techniques.

Kodachrome photographs were taken at various times of all
phases.

CHAPTER IV

Findings

I. Macroscopic Findings

At the end of the intermediate healing period, twenty one days after the initial surgery, the wound area appeared to be healed. Linear scar lines indicating the outlines of the first vestibular partial thickness flap were visible. Pigmentation, if any, within the masticatory mucosa, was not altered.

At the twenty-first day, using the scars from the previous surgery as a guide, a periosteal retention flap was raised, and was dissected away, thus leaving a large bleeding wound exposing the periosteum and some connective tissue covering the previous wound site. Hemorrhage was controlled by light pressure.

At sacrifice, the zero hour wound (Fig. 5) was covered by clotted blood. The tissue surface was pebbly and several vascular ends were seen. No vestibular alveolar bone was visible.

The first day (Fig. 7) after the re-entry surgery, the wound appeared smooth, glossy pink. There were no large blood clots, although the ends of the severed vascular channels had clots. The edges of the unoperated tissue appeared to roll into the wound.

By the second post operative day (Fig. 10) the wound appeared gray and had partly lost its smoothness. The clotted, severed vascular channels stood out clearly against the gray background.

Three days after re-entry (Fig. 12) the character of the wound had changed. A granular swollen red mass, which bled easily, was seen. The unoperated wound edges were quite distinct in contrast to the operated site.

Seven days after the periosteal retention flap had been dissected away (Fig. 14) the wound appeared to be granular with a reddish pink color. The apical portions and the interdental areas of the wound appeared to be more consistent in nature and dense as evidenced by their pink color. The central areas of the wound had a shiny, pebbly character.

The original wound borders were becoming indistinct from the unoperated tissue. The height of the gingival crests were below the tooth notch.

Fourteen days after the completion of the re-entry surgery (Fig. 17) the total wound area had the same character. The tissue appeared rather smooth, pink-red, and quite glossy.

A thin red line demarcated the operated from the unoperated sites. The crests of the gingiva were below the notches in the teeth. Clinical inflammatory characteristics were not noted.

Twenty one days after re-wounding (Fig. 19) the wound had the outward appearance of unoperated tissues. Clinical inflammation was noted in the free gingiva. The height of the gingival crests were still below the notches.

Thirty days post-operative (Fig. 21) the wounded area appeared similar to the unoperated site. However, several discrepancies were present: (a) the wound healed without a return of pigment to the tissue; (b) the wound area appeared to be denser than the unwounded area. Although marginal inflammation was present, the dento-gingival junction was considered to be totally repaired from a clinical viewpoint. In that the depth of the gingival sulci was within physiologic limits (2mm) and gentle probing of the sulci elicited no hemorrhage.

The sixty, ninety, one hundred twenty and one hundred eighty day animals (Fig. 24) were quite similar in that the wounded area appeared more dense than the unwounded area, and total clinical repair of the dento-gingival junction was evident. Clinical inflammation was present in the free gingiva; the heights of the gingival crests were at, or slightly below, the notches in the teeth; there was return of pigmentation to

pre-pigmented tissue areas.

II. Microscopic Findings

For the purpose of describing the microscopic findings the periosteal preservation wound has been divided into three major areas (Fig. 1): (a) the gingival area - the height of the gingival crest to the apical border of the epithelial attachment; (b) the supra alveolar area - the most apical point of the epithelial attachment to the crest of the alveolar bone; and (c) the alveolar area - the crest of the alveolar bone to the apical border of the wound.

A. Epithelial Repair:

At zero hour (Fig. 6) the specimen was denuded of all epithelium but portions of the epithelium remained at the gingival crest, sulcus, and the epithelial attachment. A thin blood clot had formed over the connective tissue stump adhering to the vestibular alveolar bone. There were inflammatory cells present at the gingival crest area.

Twenty-four hours after the partial thickness flap had been discarded the wound surface was covered by a thick blood clot. The coagulum had a well established fibrin network in which many polymorphonuclear leukocytes were entrapped. The epithelial attachment and remnants of the sulcular epithelium were present. Beneath the blood clot, the sulcular epithelium was seen to migrate onto and over the connective tissue

surface (Fig. 8). Mitotic figures (Fig. 9) could be seen in the basal cell layers approximately two millimeters from the wound's periphery.

Two days after re-wounding the thick clotted coagulum contained polymorphonucleocytes, and necrotic sloughing from the wound's surface. The epithelial attachment and remaining sulcular epithelium was thickened. Many mitotic figures were seen therein. The epithelium had progressed further apically at this time.

Three days post-operative the coagulus was still present with its enmeshed polymorphonucleocytes. The epithelial attachment was established at the cemento-enamel junction. The free gingiva was twelve cells thick with mitotic figures seen in the basal layers. The epithelial cells have migrated to the crest of the alveolar bone. The migrating cells taper from a four-cell thickness at the superior end to a one-cell layer at the height of the bone crest.

Seven days after the surgical re-entry (Fig. 15) the wound is completely covered with epithelium. There is no defined epithelial sulcus. The epithelium has matured as exhibited by some ridging in the supra alveolar area. A thick outer layer of keratin with parakeratosis is seen. An occasional mitotic figure is also present in the basal layer of the alveolar area. The epithelium is functional at this time for no signs of trauma to the epithelium were seen.

On the fourteenth post operative day (Fig. 17) the epithelium is ten cells thick with definite and uniform ridging. Parakeratosis is visible. There is a slight (1.0 mm) gingival sulcus. Notably, epithelial maturation is complete with definite epithelial attachment on the enamel surface.

Three weeks after re-entry (Fig. 18) the epithelium was becoming more mature. The outer surface of the epithelium was quite flat and parakeratosis was regular. The epithelial attachment is on the enamel surface. An occasional mitotic figure was seen in the basal layer of the epithelial attachment. The epithelial ridging is also quite regular.

One month after the re-entry wound procedure (Fig. 20) complete physiological and functional epithelial repair was effected. There are no significant histological or anatomic differences between this thirty-day specimen and the control.

From sixty to one hundred eighty days (Fig. 25) following the re-entry surgery, the character of the epithelium remained consistent, twelve to fifteen cells thick with regular rete peg formation. However, the amount of keratin present and the depth of the gingival sulcus was variable in each of the specimens. The epithelial attachment remained the same, both in character and in position on the tooth's enamel. An inflammatory process was visible within the subepithelial connective tissue in all these later animals.

B. Connective Tissue Repair:

The zero hour specimen (Fig. 6) showed a definite layer of connective tissue and a fibro-periosteum covering the vestibular alveolar bone. An inflammatory response was evident in the supra crestal deep lamina propria, capillary dilation, marginazation and extra capillary migration of white blood cells. Many polymorphonucleated cells and lymphocytes were present underneath the clot along the edge of the wound. The supra crestal collagen bundles were disorganized. Parallel to the vestibular plate of bone, a linear, well-organized, dense periosteum was present. The periodontal ligament space was compatable in its width to the control; although the capillaries in the supra crestal regions were engorged with cells, mature collagen fibers could be seen. There was no evidence of the vestibular alveolar bone having been injured by the surgical manipulations.

The periosteal preservation tissue at twenty-four hours (Fig. 8) showed a definite inflammatory response with many dilated capillaries, especially toward the periodontal ligament. Extravasated red blood cells were seen directly underneath the organized blood clot. Also, directly under the coagulum, the collagen fiber bundles were disorganized with edematous material between the fibers. The Supra crestal collagen fibers and the periodontal ligament fibers were not disorganized and appeared thickened. The cambium layer of the periosteum had

an increased cell population, mononuclear connective tissue cells. At three days post operative, the disorganization of the collagenous fiber bundle was still present. The capillaries within the connective tissue surface were also still enlarged. However, many perivascular reserve-type cells were seen undergoing mitosis. Many red blood cells and connective tissue cellular elements were seen interspersed throughout the disorganized fiber bundles. No necrotic elements were noted.

By one week (Fig. 15) the ratio of connective tissue cellular elements to collagenous material had increased. There was a decrease in the size and number of capillaries, both in the connective tissue surface and in the periodontal ligament space. There was also a decrease in the number of polymorphonucleocytes in the super crestal and alveolar wound areas. However, a large number of this type of cell was seen immediately beneath the repaired gingival crest. Although the collagen fiber bundles were still disorganized, it was not as great as in the three-day specimen. Also, new collagen was being elaborated by newly differentiated fibroblasts.

Although a few plasma cells were seen in the superficial lamina propria at two weeks (Fig. 18) there were very few inflammatory type cells seen. The ratio of connective tissue cells to collagen had greatly decreased. Well organized collagen bundles were formed at this time. Those collagen

bundles immediately adjacent to vestibular alveolar bone were more darkly stained than those bundles in the superficial lamina propria.

Three weeks after the re-entry surgical procedure (Fig. 20) the connective tissue attachment to the tooth had increased. The collagen bundles were maturing and the amount of inflammatory cells present underneath the gingival crest was compatible to the control specimen.

By one month after re-wounding (Fig. 22) maturation of the wounded connective tissue was accomplished. The control specimen and the thirty day experimental specimen were quite compatible. Allowing for species variation of the investigative animals the sixty-day, ninety-day, one hundred twenty day and one hundred eighty day specimens (Fig. 25) show the same mature connective tissue pattern.

However, the special connective tissue stains employed (trichrome and silver impregnation) show the connective tissue bundles to be more dense, for they both impart to the repaired connective tissue a more heavy stain than have the unwounded areas, and they emphasize how closely and regularly aligned are the collagen fiber bundles.

C. Osseus Reaction:

The zero hour specimen (Fig. 6) showed the effects of the first wound. A reversal line, indicating osteoclasia followed by osteogenesis, was evident beneath the scalloped edges of the vestibular plate. Many osteoblastic cells were seen in

the cambium layer of the periosteum. Beneath the periosteum, osteod material was present in which there were entrapped osteoblasts. Osteogenesis was also seen on both sides of the vestibular alveolar crest. There was no evidence of osteoclastic resorption or osteoclasts on the periodontal ligament side of the vestibular plate. Nor were any empty osteocytic lacunae seen. However, a heavy layer of cementum and cemento-genesis was noted on the root surface of the tooth in the investigative area. The distance from the height of the alveolar crest to the lower edge of the tooth notch was measured to be 9.33 mm (Table IV).

Twenty-four hours after the re-entry wound, osseus contour was similar to that present at zero hour. However, an increase of cell density was seen within the crestal marrow spaces on the vestibular crest and crestal periodontal ligament surfaces of the vestibular alveolar bone. Empty osteocytic lacunae were not seen. The distance from the height of the alveolar crest to the lower edge of the tooth notch was measured to be 9.32 mm.

By two days (Fig. 11) osteoclasts are seen at the alveolar crest from both the vestibular and periodontal ligament sides, but they are still within the fibroperiosteum and away from the alveolar bone surface. Engorged capillaries are seen within the marrow spaces. Some of the vestibular surface marrow spaces are continuous with the periosteal retention area. A scalloped vestibular border with some osteogenic activity is

still present. No empty lacunae were noted. The distance from the height of the alveolar crest to the lower edge of the tooth notch was measured to be 9.37 mm.

The third post-operative day (Fig. 13) showed osteoclastic activity on the vestibular surface, and within the vestibular marrow spaces. Howship's lacunae were also present on the periodontal ligament side of the alveolar crest. Further apically within the periodontal ligament enlarged and cellular engorged capillaries were present. The cambium layer of the periosteum was quite cellular, mononuclear connective tissue cells. There was no evidence of instrumental bone damage. The distance from the height of the alveolar crest to the lower edges of the tooth notch was measured to be 9.46 mm (Table IV).

From the third to the seventh post operative day (Fig. 16) there had been considerable osteoclastic activity. The seven day re-wounded specimen showed nests of osteoclasts within the Howship's lacunae, all on the vestibular surface. There was a visible decrease in the vertical and horizontal dimension of the alveolar bone crest. However, within the periodontal ligament space, there was seen osteoblastic activity at the bone crest. No osteoclastic activity in this area was noted. There was also a decrease in the number and size of the capillaries within the periodontal ligament space. The distance from the height of the alveolar crest to the lower edge of the tooth notch was measured to be 10.65 mm which was a marked

reduction in alveolar crest height; there were no empty osteocytic lacunae seen.

Fourteen days after the re-entry surgery (Fig. 18) there was a continuous scalloped reversal line on the vestibular surface. Only two osteoclastic nests were seen on the vestibular surface. The marrow spaces had decreased in size and appeared void of osteoclastic nests. At the crestal portion of the periodontal ligament, osteoid tissue and an increased cell population was seen. The distance from the height of the alveolar crest to the lower edge of the tooth notch was measured to be 10.63 mm, which is also a significant loss in crestal bone height.

By the twenty-first day after re-entry (Fig. 20) the mononuclear cell density had greatly increased at the bone crest within the marrow spaces. Osteogenesis was present on all alveolar bone surfaces, with the vestibular surface having the most activity. Nests of osteoblasts, six on the periodontal ligament side and four on the vestibular surface, were present in a chain-like fashion near osteoid tissue. There were no osteoclasts or osteoclastic action noted. The distance from the height of the alveolar crest to the lower edge of the tooth notch was measured to be 8.72 mm.

One month after the second surgical procedure (Fig. 22) osseous reaction to the periosteal preservation re-entry was practically complete. A definite reversal line was visible at the level of the alveolar crest. The marrow spaces were

of the same size as the control and minimal or no cellular activity was seen. The vestibular periosteum (Fig. 23) was three cell layers thick with the inner layer having the greatest density of cells. A few osteoblasts were present at the alveolar crest and the maturation of previous osteoid and the elaboration of osteoid was present on the vestibular surface and periodontal ligament side of the alveolar bone. No osteoclasts were seen and the periodontal ligament space was normal. The distance from the height of the alveolar crest to the lower edge of the tooth notch was measured to be 9.30 mm. This distance is identical to the one measured in the control animal.

By the end of two months continued maturation of osteoid tissue was evident. Osteogenesis was still progressing at the alveolar crest and on the vestibular surface with osteoblasts elaborating new material.

Bundle bone was present on the periodontal ligament side of the alveolar bone. The crestal bone was increasing in thickness, with both circumferential and laminar bone present. The distance from the height of the alveolar crest to the lower edge of the tooth notch was measured to be 9.54 mm.

Three months after surgical re-entry, osteogenesis was still evident at the alveolar crest on the vestibular surface and on the periodontal ligament crestal surface. The new laminar bone laid down on the vestibular surface appeared to be quite dense. The distance from the height of the

alveolar crest to the lower edge of the tooth notch was measured to be 9.46 mm.

At four months, lessened osteogenic activity was seen on the periodontal ligament crestal surface. Although a definite reversal line was present at the alveolar crest, osteogenesis was still evident in the specimen at the crest. Osteogenesis was also seen on the vestibular alveolar surface. The distance from the height of the alveolar crest to the lower edge of the tooth notch was measured to be 9.33 mm.

The last experimental specimen was sacrificed six months after the periosteal preservation wound was made (Figs. 25, 26). Osteoblasts were still present at the alveolar crest and on the periodontal ligament side of the crest. The cambium layer of the periosteum was still quite cellular. The crestal bone was blade-like with new bone being evident, superior to a definite elliptical reversal line. On the periodontal ligament side of the crest the embedding of Sharpey's fibers in the new crestal bone gave the bone a laddered appearance. The maturation of the new osseous material was comparable with the control bone. The distance from the height of the alveolar crest to the lower edge of the tooth notch was measured to be 7.78 mm.

CHAPTER V

Discussion

I. Introduction.

The discussion of the presented experimental findings is predicated upon these assumptions: anatomic variations were within acceptable biologic limits; the same biologic healing and repair processes occurred to the same degree within each of the experimental animals; and the amount of remaining periosteal preservation tissue was identical in each of the experimental animals. These assumptions were made so that a cohesive interpretation of the investigative findings could be formulated. However, it must be recognized that no allowance has been made for animal variation since no accurate determination of the quantitative and qualitative degree of genetic variation within the species is assessable.

It is theorized that at the time of re-entry surgery: (1) there has been an increase in the number of connective tissue reserve cells within the fibroperiosteum, and particularly in those connective tissue reserve cells which have differentiated and are differentiating into osteoblasts and fibroblasts; (2) these differentiating cells continue to produce their products which, in turn, protects to a degree the alveolar

bone from the trauma of a second injury by their availability prior to injury; (3) the re-entry surgery induces the connective tissue to produce more reserve cells instead of remaining in a "resting" stage. This is especially true with respect to the reserve cells that will differentiate into osteoblasts and fibroblasts. Therefore, the end result of re-entry surgery will produce a more rapid healing wound with less trauma to the supporting alveolar bone. The mechanism which determines the destiny of a connective tissue reserve cell is unknown at this time. } 206 37

II. Role of the Inflammatory Process.

The biologic phenomenon of the inflammatory process serves as a source of cells available for the protection of the wound site, and as a means to prepare the remaining periosteal preservation tissue for repair. The latter facet of inflammation is of particular interest. It seems logical that before cells can be mobilized for repair, space must be provided for them. It was seen that the densely packed collagenous fiber bundles adjacent to the fibroperiosteum became disorganized and irregularly arranged due to the inflammatory exudates, which in effect created the needed space for the cells.

Concomitantly with the disorganization of the remaining lamina propria, the severed ends of the capillaries proliferate to provide channels through which cellular nutriments are provided and to bring more inflammatory cells to protect the open



wound. The speed of this reaction is then dependent upon the preponderant availability of cells to be mobilized.

III. Epithelial Repair.

The rate with which the epithelial cells migrate to cover an open wound is significant. For the rate is a measure of the receptiveness of the tissue the epithelium covers, in this instance, whether or not the connective tissue is vascular enough to provide nutrients to the migrating epithelium, and if the connective tissue is free of noxious materials. Once the proper environment is established, then the size of the space to be transversed and the source of epithelial cells available becomes important. The source of available epithelial cells was already present; the epithelial cells adjacent to the wound's periphery were the ones that provided cells for migration under the coagulum to cover the injury. The defect provided by the re-entry procedure was quite large, 43 mm by 10 mm, and so it would require a rather long period of time for complete wound coverage. However, the periosteal preservation re-entry wound was observed to be completely covered in seven days with a parakeratotic epithelium which means that the earliest closure of the wound would occur at some time on or about the fifty day after re-entry. This time-table of epithelium coverage and maturity is the most rapid yet reported for a gingival wound of this size and type.

IV. Connective Tissue Repair.

The majority of mature connective tissue was removed by virtue of discarding the soft tissue flap at the time of re-entry so that there was a minimal amount of connective tissue elements available in the remaining lamina propria and periosteum for repair.

It is well established that the un-differentiated reserve connective tissue cells are the cells that differentiate into fibroblasts for connective tissue repair, as is supported by Toto and Ramfjord. The reserve cell source was seen to be the alveolar bone complex: (1) fibroperiosteum, (2) alveolar bone proper, and (3) periodontal ligament. The fibroperiosteum had an increased number of reserve cells in mitosis and differentiating as a result of the first injury. This initial preparedness is the major source of reserve cells due to the early conditioning of the wound site. The rate with which repair is effected is dependent upon the number of reserve cells present. In this study, connective tissue repair was comparatively rapid. Therefore, the quick and local availability of cells was no doubt a factor in its repair. It is also thought that the differentiation of the reserve cells into fibroblasts is a local phenomenon; that as new capillaries proliferate, the reserve cells migrate along with them; and that this process occurs first adjacent to the fibroperiosteum in the alveolar area, the periodontal ligament, and untraumatized super crestal

lamina propria in the gingival area. Then the continuing process of capillary proliferation with accompanying reserve cell mitosing and/or differentiating proceeds towards the outer portion of the wound.

Fibroblasts elaborate tropo collagen as their product; and tropo collagen then matures into collagen. The more fibroblasts present would presume a larger quantity of their product, and it is thought that the more collagen that is available, the denser the connective tissue would become. It was observed that the special connective tissue stains employed showed dense collagen. It is also thought the arrangement of maturing collagen into definite oriented bundles is not only dependent upon the functional stress placed upon the connective tissue and in a larger sense upon the periodontium, but is also dependent upon the status of alveolar bone complex. An apically positioned dento-gingival junction supposes an alveolar bone that is deficient in height, as was discussed by Wilderman (1959, 1963) and Staffileno (1966).

V. Osseus Reaction.

It is well documented that alveolar bone is affected in any gingival surgical procedure, and the amount of reaction is dependent upon the severity and length of the surgical procedure. How then can the alveolar bone be protected if a severe surgical procedure is intended?

Giblin (1966) indicated a method for alveolar bone protection by utilizing a double wounding procedure. The information obtained from Giblin's work was then adapted to this more severe second wound.

It is thought that the best protection for a biologic tissue is the protection it gives itself. A primary less severe wound initiates a well documented osseus reaction, osteoclasia, followed by osteogenesis, a thickening of the periosteum and a mobilization of reserve cells. It is these biologic processes that are utilized for protection from the second extremely traumatic wound. How then do these biologic processes work to limit detrimental osseus reactions?

(Theoretically it is thought: ^{from Giblin's studies that:} (1) The fibroperiosteum acts as a barrier to the effects of the inflammatory process within the remaining lamina propria; (2) the osteoid which was laid down as a reaction to the first injury also acts as a barrier to the osteoclasia resulting from the second injury, thus protecting the uninjured alveolar bone; (3) the reserve cells already mobilized as a result of the first injury continue to be active in differentiating into osteoblasts; (4) the reserve cells that differentiate into osteoclasts compete in activity with the osteoblasts so that the net cellular activity results in a minimized trauma to the supporting alveolar bone.

The process of minimized trauma to the supporting alveolar bone is made clear by an analysis of the measurements (Table IV) made from the crest of the alveolar bone to the lower border of

the tooth notch. The control measurement was 9.30 mm which represented an un-traumatized-physiologic alveolar bone. At the time of re-entry (twenty-one days after the initial injury) there was a 0.03 mm loss in the height of the bone. This represented osteoclasia followed by osteogenesis. Following re-entry, there was a gradual decrease in the alveolar bone height until the twenty-first day. At this time period, the height of osteoblastic activity following rewounding, the continuing osteogenesis, showed an increase of 0.58 mm in bone height from the control specimen. It is thought that this can only be due to a preponderance of osteoblastic activity which in turn supposes a great mobilization of cells and that the cells had to be already present in the area to be mobilized. It is biologically unreasonable to suppose a tissue is able to mobilize its cells anew to produce this type of physiologic activity in such a short span of time.

The return to the control bone height by the thirtieth day after re-entry is thought due to a return of the alveolar bone to its genetic norm, and the maintenance of this norm over the succeeding months.

As we compare this method of wounding with that of Wilderman (1963) it is observed that this procedure produces lesser traumatic effects during repair process. The type and size of the wounds are exactly the same. However, the re-entry wound has initially established a cellular mobilization within the therapeutic wound site so that the detrimental trauma of

the second wound is barely recorded upon the tissues. It is observed, however, that the two similar periosteal preservation wounds repaired with a marked difference in the quality of repair. The rapidity of repair in the re-entry wound is without a doubt due to the preparedness of the original wound site at both the cellular and "humoral" level. This preparedness establishes the presence of rapidly available reserve cells, fibroblasts and osteoblasts, all of which are needed in order to complete a rapid repair.

The process of epithelization is seen to occur approximately four days earlier than in Wilderman's (1963) study. There is a greatly reduced osteoclasia and rapid osteogenesis, all of which serves to demonstrate the effectiveness of preparing a tissue site for repair even previous to contemplated wounding of an extensive and traumatic nature such as occurs in periosteal retention type wounds.

The most significant occurrence of a re-entry periosteal preservation type procedure is the rapidity of repair with a minimal amount of osseous resorption and actual osseous protection that is afforded the various phases of the repair process.

CHAPTER VI

Summary and Conclusion

A vestibular partial thickness flap measuring 10 mm by 43 mm, was reflected from the mandibular pre molar teeth in six mongrel canines. The flap thus raised was immediately reapposed to its original position. This primary surgical wound was allowed to heal for twenty one days. At the end of the intermediate healing period, the same partial thickness flap was again reflected and discarded, leaving a large periosteal preservation wound to heal and repair. The experimental animals were sacrificed at specified time intervals following re-entry surgery. The following were the major observations:

1. Complete maturation of the dentogingival junction without deformity occurred by the twenty first day, and complete repair of the periodontal complex was achieved by the thirtieth day after re-entry surgery.

2. Microscopically, the epithelium was seen to begin migration the first day following re-entry and

to completely cover the wound with mature epithelium in seven days.

3. Following initial surgery, there was osteoclastic resorption followed by osteogenesis.

4. Following initial surgery there was present a fibroperiosteum.

5. Following the re-entry, periosteal preservation procedure, there was osteoclastic activity superimposed upon osteoblastic activity, so that the loss of alveolar bone was held to a minimum, 0.03 mm.

6. Osteoclastic activity reached its peak by the seventh day following re-entry. The total loss of bone in the seven days following re-entry was 1.32 mm.

7. Osteoblastic activity reached its peak by the twenty first day following re-entry. The gain of bone in the twenty one days following re-entry was 0.58 mm.

8. Osteogenesis was present at the time of re-entry and continued until the completion of the investigation. A net increase in the height of bone from the thirtieth day to the end of the experiment following re-entry was measured to be 0.22 mm.

From the noted observations it is concluded that this study supports the hypothesis that re-entry periosteal preservation surgery results in:

- (a) A re-entry wound which heals and repairs in the same sequence as the first wound.
- (b) Expeditious repair of the periodontal complex.
- (c) Maximum preservation of the supporting alveolar bone.

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TABLE ISummary of Epithelial Repair

"O" Hour	<ol style="list-style-type: none"> 1. Remnants of sulcular epithelium and epithelial attachment present. 2. No other epithelial activity.
Days 1-5	<ol style="list-style-type: none"> 1. Mitotic activity in basal layers. 2. Apical growth of epithelium under coagulum, and over connective tissue surface. 3. Necrotic sloughing of cells and cellular debris.
Days 6-21	<ol style="list-style-type: none"> 1. Periosteal preservation wound completely covered with epithelium. 2. Re-establishment of the epithelial attachment and maturation of epithelium with elaboration of keratin.
Days 22-30	Further maturation of epithelium with complete physiological and functional repair seen at 30 days.
Days 31-180	Maintenance of mature epithelium and influx of gingival inflammation present in the experimental animal.

TABLE II

Summary of Connective Tissue Repair

"0" Hour	<ol style="list-style-type: none">1. Connective tissue stump consisting of deep lamina propria connective tissue and periosteum.2. Disorganization of the fiber bundles in connective tissue stump.3. Inflammation in the connective tissue stump.4. Periodontal ligament space normal.
Days 1-5	<ol style="list-style-type: none">1. Extravazation of red blood cells into connective tissue spaces.2. Inflammatory reaction in periosteal preservation wound and periodontal spaces with continued disorganization of connective tissue fiber bundles.3. Increase in perivascular and periosteal cellular activity in the supra crestal area and on the vestibular and periodontal ligaments side of the alveolar bone.
Days 6-21	<ol style="list-style-type: none">1. Decrease and elimination of the inflammatory response elaboration of collagen with a marked decrease in the cell to cellular product ratio.2. Maturation of collagen starting at vestibular bone surface and progressing to epithelium.3. Increase in length of the connective tissue attachment.
Days 22-30	<ol style="list-style-type: none">1. Completed maturation of collagen fiber bundles.2. Physiological and functional repair of the dentogingival junction.
Days 31-180	Maintenance of the mature connective tissue and re-introduction of gingival inflammatory response normally seen in the experimental animal.

TABLE III

Summary of Osseus Reaction

"O" Hour

1. Evidence in the vestibular portion of the alveolar bone of osteoclasia followed by osteogenesis.
2. No instrumental damage seen to the alveolar bone.

Days 1-13

1. Osteoclastic activity which decreased the height and vestibular breadth of the alveolar bone.
2. Periodontal ligament space osteoclasia limited to the crestal area.
3. No evidence of instrumental damage to the alveolar bone seen.
4. Peak of osteoclast activity seen at 7 days.
5. In all time periods some osteoblastic activity present.

Days 14-30

1. Cessation of osteoclastic activity with continued osteoblastic activity which reached a peak at 21 days.
2. Total repair of alveolar crest without deformity.

Days 31-180

Continued osteogenesis and maturation of osseus material.

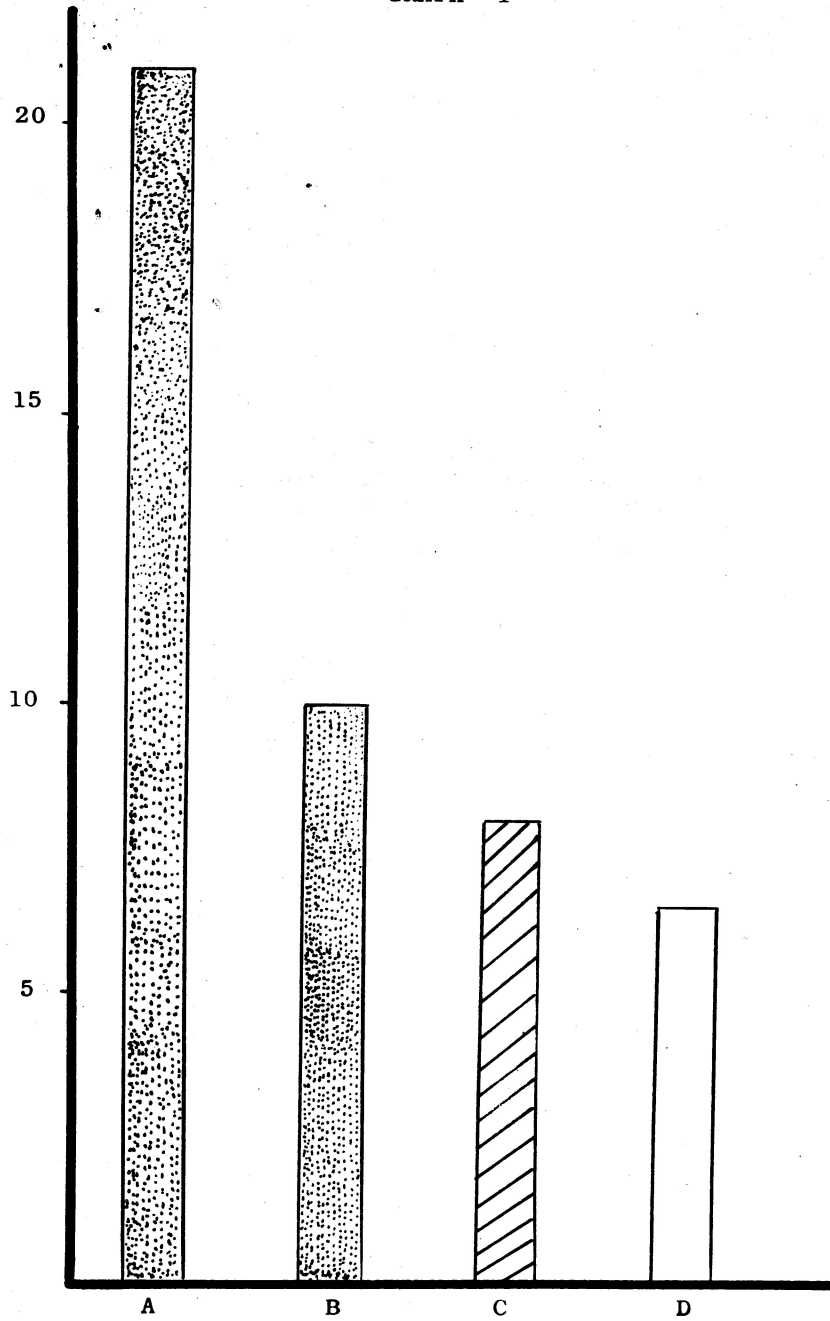
TABLE IV

Interval from re-entry
surgery to time of sac-
rifice.

Average distance from the height of
the alveolar bone crest to the lower
border of the notch in the tooth
measured in mm.

Control	9.30 mm
Zero Hour	9.33 mm
One day	9.32 mm
Two days	9.37 mm
Three days	9.46 mm
Seven days	10.65 mm
Fourteen days	10.63 mm
Twenty one days	8.72 mm
Thirty days	9.30 mm
Sixty days	9.54 mm
Ninety days	9.46 mm
One Hundred Twenty days	9.33 mm
One Hundred Eighty days	7.78 mm

GRAPH 1

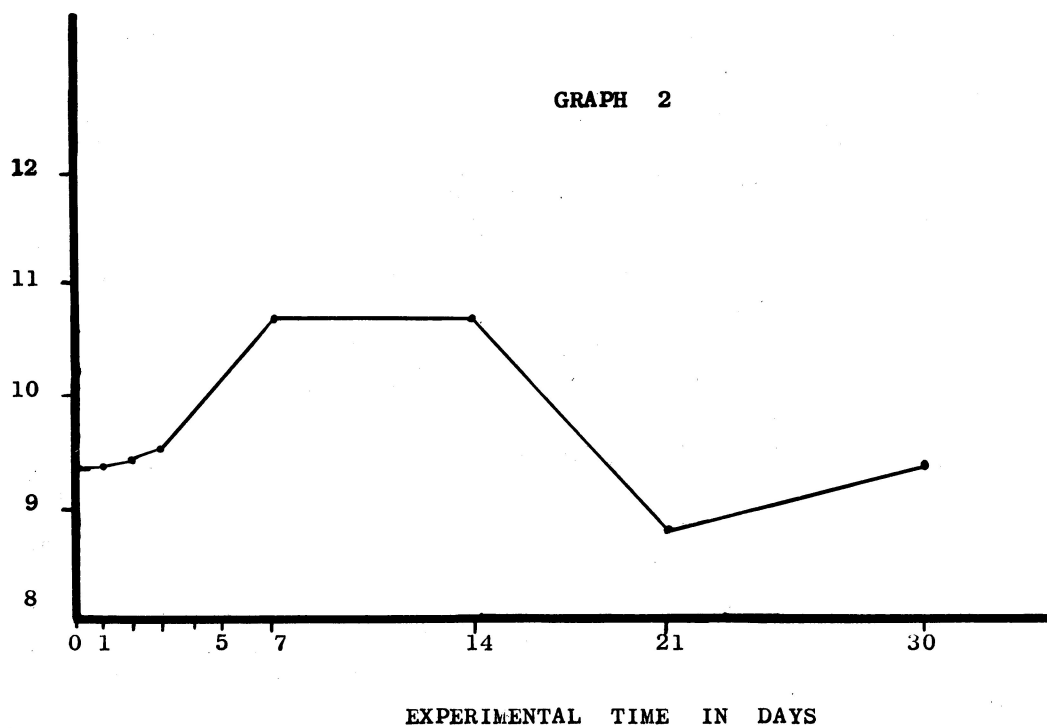


GRAPH 1

Comparison of the Differences in the Rate
of Re-epithelization of Periosteal
Preservation Wounds.

- | | |
|------------------|------|
| A. Wilderman | 1959 |
| B. Wilderman | 1963 |
| C. Staffileno | 1966 |
| D. Present Study | 1967 |

GRAPH 2



GRAPH 2

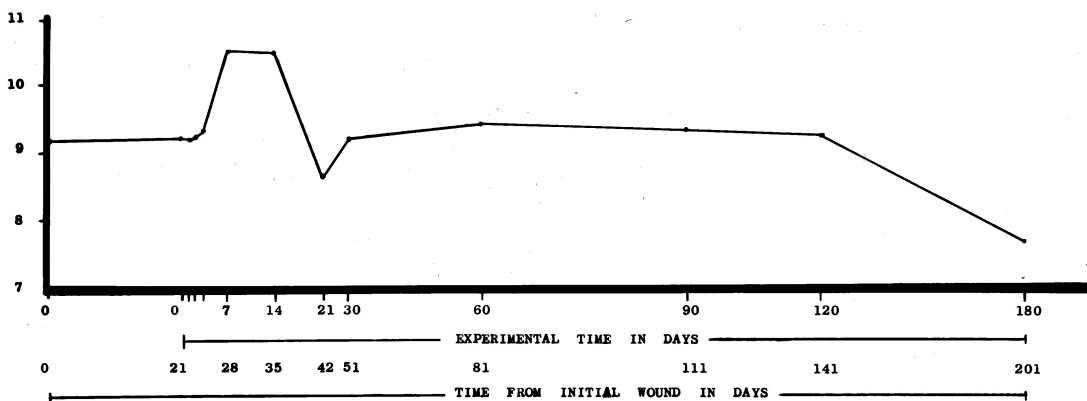
The plotting of the osseus reaction occurring during the first thirty days following the re-entry procedure. The abscissa is expressed in days while the ordinate is expressed in millimeters.

For exact measurements refer to Table IV.

NOTE:

- A. Height of osteoclastic activity at seven days.
- B. Height of osteoblastic activity at twenty one days.

GRAPH 3



GRAPH 3

The plotting of the osseus reaction occurring during the total time of the investigation. The abscissa is expressed in days while the ordinate is expressed in millimeters. For the exact measurements refer to Table IV.

FIGURE 1

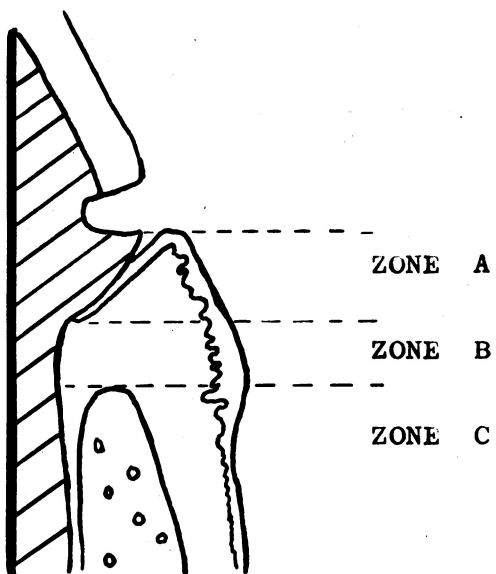
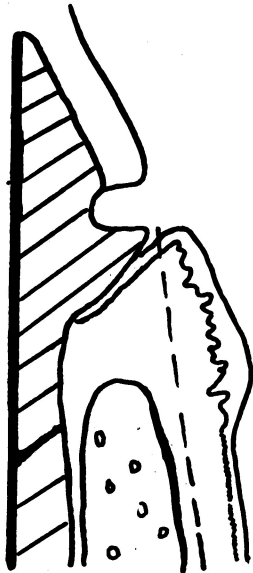


Figure 1:

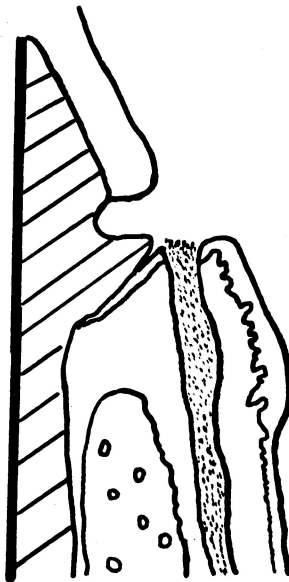
Illustration of the anatomic relationships of the periodontal complex. For purposes of description, the investigative wound area has been divided into three areas.

- | | |
|------------------|--------------------------------|
| 1. Zone A | The Gingival Area |
| 2. Zone B | The Supra Gingival Area |
| 3. Zone C | The Alveolar Area |

FIGURE 2



A



B

Figure 2:

Two illustrations of the investigative wounding procedures.

- A. The dotted line indicates the path of the surgical incision used during both surgical entries.
- B. The stippled area indicates the blood clot which formed over the wound site. The tissue to the right is the tissue that is to be discarded. The tissue to the left is the periosteal preservation wound.



Figure 3.

**Clinical Photograph of the preoperative
pre molar area.**

Figure 4.

Photomicrograph of the control periodontium 100

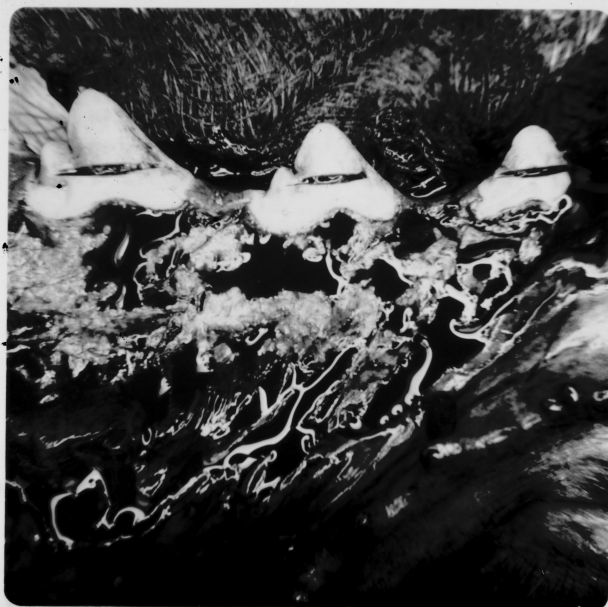


Figure 5:

Clinical photograph of the zero hour wound

Figure 6:

Photomicrograph of the zero hour specimen. 100X

- 1. Blood clot on the wound's surface.**
- 2. Scalloped vestibular margin with a definite reversal line.**

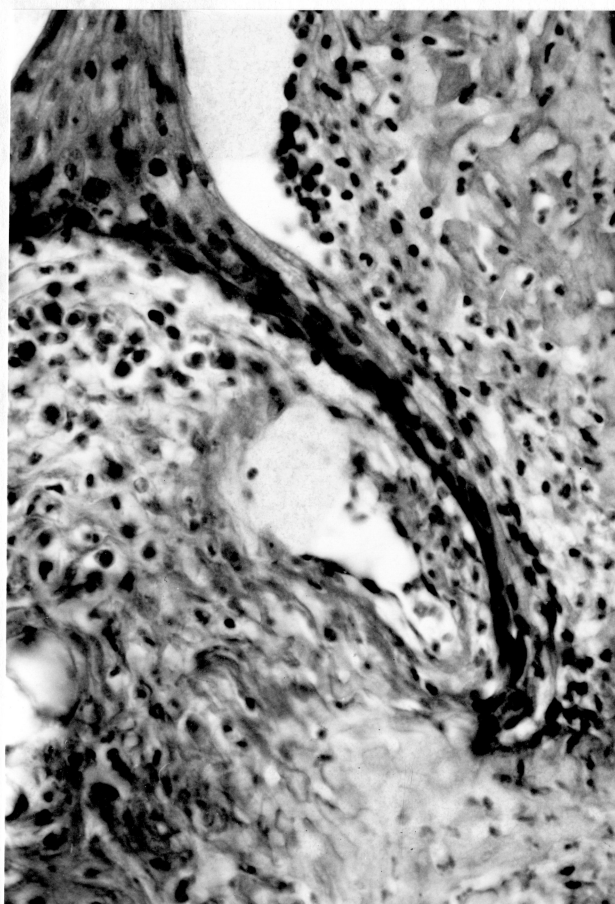


Figure 7:

**Clinical Photograph of the one-day post
operative re-entry specimen.**

Figure 8:

**Photomicrograph of the one-day post
operative re-entry specimen.**

**Downgrowth of epithelium beneath the
coagulum. 450X**

- 1. Single cell layer of downgrowing
epithelium.**
- 2. Enlarged capillary with marginization
of cells beneath the downgrowing epithelium.**

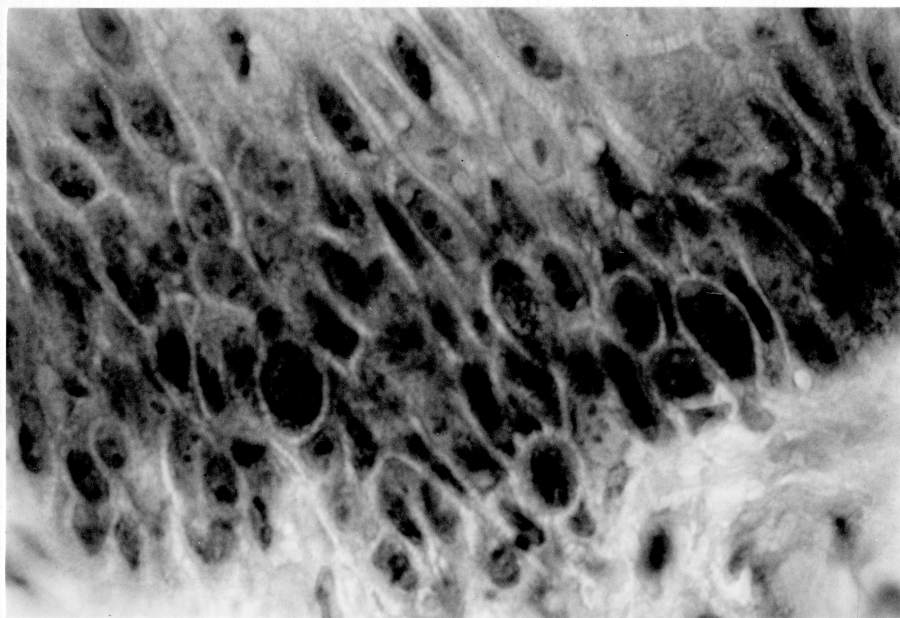


Figure 9:

Photomicrograph of the one-day post operative
re-entry specimen.

Mitotic figures at the basal cell layer of
the epithelium. 1000X

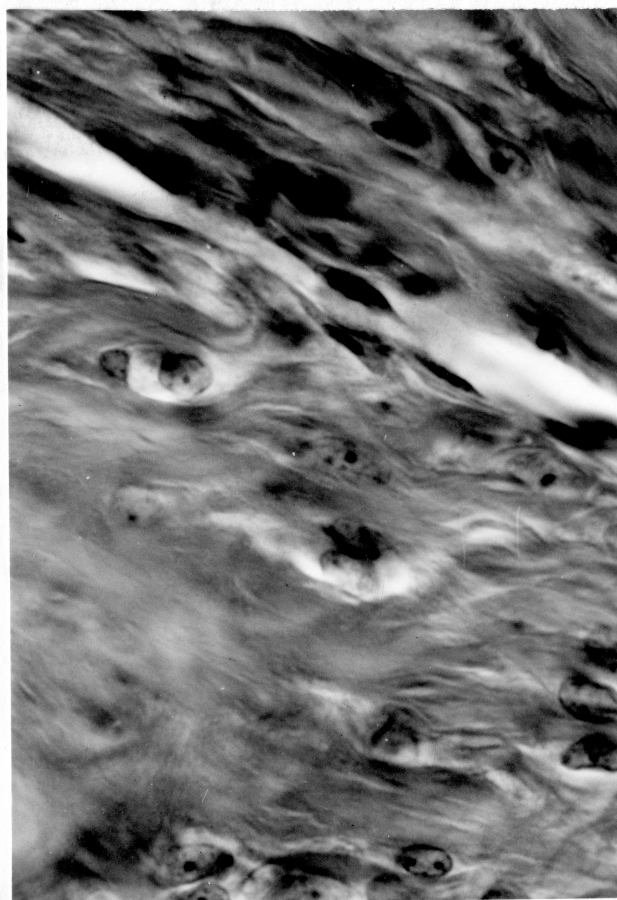


Figure 10:

**Clinical Photograph of the two-day post
operative re-entry specimen.**

Figure 11:

**Photomicrograph of the two-day post operative
re-entry specimen.**

**Cells within the cambium layer of the fibro-
periosteum. 1000X**

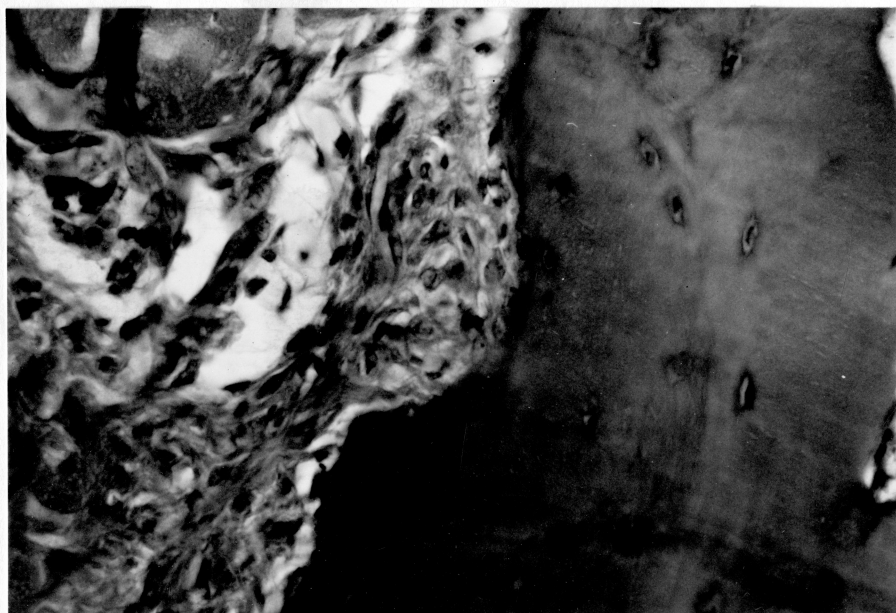


Figure 12:

**Clinical photograph of the three-day post
operative re-entry specimen.**

Figure 13:

**Photomicrograph of the three day post operative
re-entry specimen.**

**Osteoclasts within Howship's lacunae on the
vestibular surface of the supporting alveolar
bone. 450X**



Figure 14:

Clinical Photograph of the seven day post
operative re-entry specimen.

Figure 15:

Photomicrograph of the seven day post
operative re-entry specimen.

Zones A, B, and C of the wound. 100X

Figure 16:

Photomicrograph of the seven day post
operative re-entry specimen.

Zone C of the Wound. 100X

1. Osteoclastic activity on the vestibular surface of the alveolar bone.
2. Osteogenesis on the periodontal ligament surface of the alveolar crest.



Figure 17:

**Clinical Photograph of the fourteen day
post operative re-entry specimen.**

Figure 18:

**Photomicrograph of the Fourteen day post
operative re-entry specimen.**

Zones A, B, and C of the wound. 40X

- 1. Return of the gingival sulcus**
- 2. Organization of the connective
tissue fiber bundles**
- 3. Reversal line on the vestibular surface**

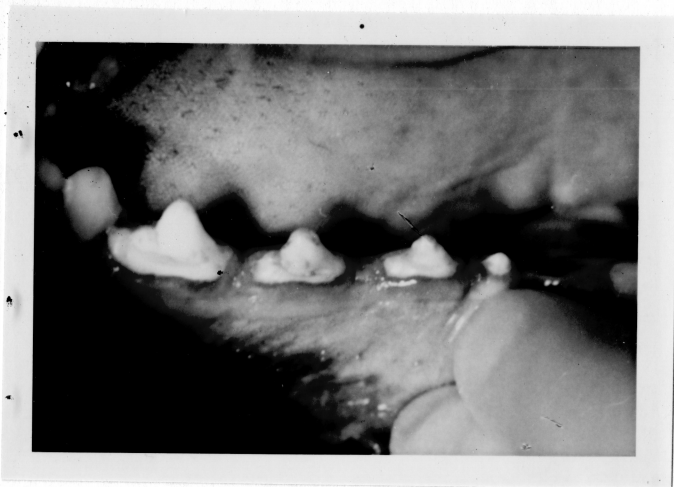


Figure 19:

Clinical photograph of the twenty-one day post operative re-entry specimen.

Figure 20:

Photomicrograph of the twenty-one day post operative re-entry specimen.

Zones A, B, and C of the wound. 40X

1. Inflammation underneath the free gingival margin.
2. Osteogenesis on the vestibular surface of the alveolar bone.



Figure 21:

Clinical photograph of the thirty-day post
operative re-entry specimen.

Figure 22:

Photomicrograph of the thirty-day post
operative re-entry specimen.

Zones A, B, and C of the wound. 40X



Figure 23:

Photomicrograph of the thirty day post
operative re-entry specimen. 100X

1. Cellularity of the cambium layer of the periosteum.
2. Osteogenesis on the vestibular surface of the alveolar bone.

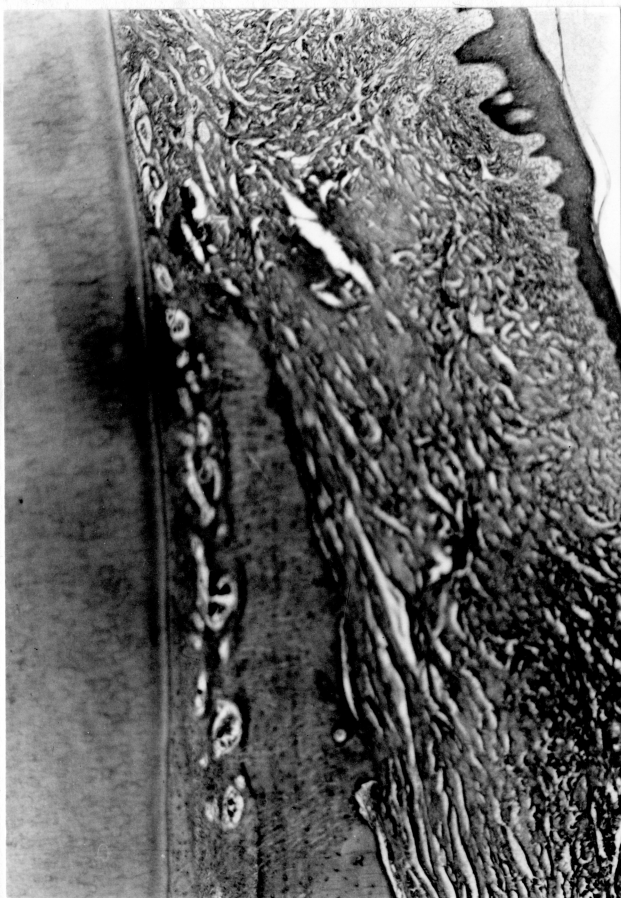
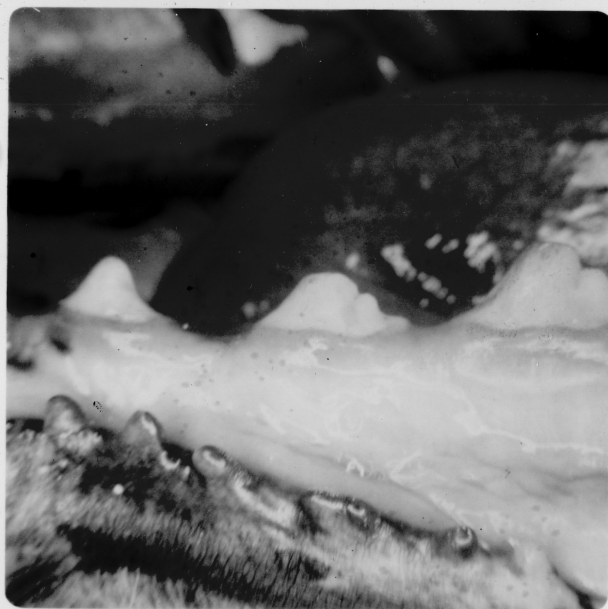


Figure 24:

Clinical photograph of the one hundred eighty day post operative re-entry specimen.

Figure 25:

Photomicrograph of the one hundred eighty day post operative re-entry specimen. 40X

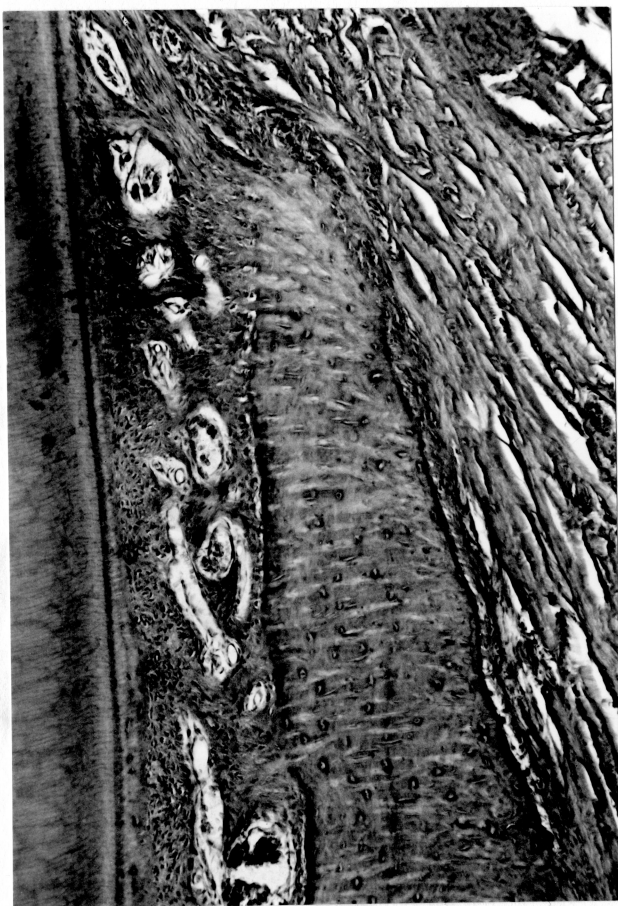


Figure 26:

Photomicrograph of the one hundred eighty day post operative re-entry specimen. 100X

1. Osteogenesis at the alveolar crest.
2. Embedded Sharpy's fibers on the periodontal ligament side of the alveolar bone.

Approval Sheet

The thesis submitted by Dr. Peter Dittmar Roberson has been read and approved by three members of the faculty of the Graduate School.

The final copies have been examined by the Director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

5-24-67

Date

Anthony W. Longino

Signature of Advisor